

Effect of Sampling Time and Educational Status of the Lactating Mothers on Human Milk Compositions

R. Khatun^{1,*}, S. Akter¹, M. M. Ahasan², M. F. Uddin², M. R. Islam², H. M. Jamil², A. N. Monika²,
A. Rahman², M. Rahman³, R. P. Das², R. A. Sharmin², M. Ohiduzzaman⁴

¹Medical Physics Division, Atomic Energy Centre, Dhaka, Bangladesh

²Institute of Nuclear Medical Physics, AERE, Savar, Dhaka, Bangladesh

³Accelerator Facilities Division, Atomic Energy Centre, Dhaka, Bangladesh

⁴Department of Physics, Jessore University of Science and Technology, Jessore, Bangladesh

Abstract Breast milk is the best source of nutrition for the newborn infant. There are several factors which may affect the human milk compositions. Among them sampling time and educational status of the lactating mothers is the core interest in this study. In almost all countries, child-death rates are inversely related to the level of maternal education. The study has been conducted to observe whether the human milk varies with sampling time and educational status of the sample providing lactating mothers. The experiments have been done with the 3 MV horizontal type (KN 3000) positive ion Van de Graaff accelerator at the Atomic Energy Center, Dhaka (AECDC) by using Ion Beam Analysis (IBA) technique, PIXE. The quantitative measurement of trace elements in the present study is as follows: S 2493 ± 530 ppm, Cl 11204 ± 1669 ppm, K 14334 ± 2369 ppm, Ca 10383 ± 1493 ppm, Fe 60.24 ± 21.61 ppm, Cu 6.23 ± 3.11 ppm, Zn 30.3 ± 15.20 ppm, Br 7.54 ± 4.65 ppm and Rb 13.4 ± 7.71 ppm. The concentrations of the elements in the afternoon (PM) samples are obtained higher than those in the morning samples (AM) and concentrations of Zn, Fe and Ca content of breast milk from mothers having educational status Secondary School Certificate (SSC) and above was significantly higher than those of illiterate and primary educated mothers. The measured values of obtained elements are compared with the certified values of Standard Reference and found to be compatible. This article provides an overview of the composition of human milk and sources of its variation pattern with them.

Keywords Human milk, Nutrients, Toxic elements, PIXE

1. Introduction

The main pathway of the elements to the human body is the food. Human milk is the most important common food of babies during the early years of their life all over the world. Breast milk is the best source of nutrition for the newborn infant, and exclusive breast feeding is recommended up to 6 months of age [1]. In general, breast milk contains all nutrients required for proper infant development. Even though human milk is the best baby food, a large number of babies die annually as a result of malnutrition and nutritional deficiencies. Recently some major environmental problems are encountered in Bangladesh related to the presence of a number of toxic

elements like arsenic and fluoride in ground water and lead in air [2-3]. Physicians in Dhaka Shishu Hospital have found lead in bloods of some babies and Zinc deficiency in arable soil has also become a serious problem [4]. In this situation, the high mortality rate of children in Bangladesh is a great concern and hence search is needed to find out ways and means to reduce the child mortality rate. Women's education in particular is the most powerful determinant of trends in public health and child mortality. The correlation between parental education and child mortality has been extensively documented. In almost all countries, child-death rates are inversely related to the level of maternal education. The more educated the mother, the healthier she and her child are likely to be. Comparative research focused on 33 countries during the 1980s found that each additional year of maternal education reduced childhood mortality by about 8% [5]. Besides them sampling time is also a parameter which may the concern of nutritional composition of human milk.

Several factors, such as dietary intake and genetic background, influence the elemental composition of human

* Corresponding author:

rajada_75@yahoo.com (R. Khatun)

Published online at <http://journal.sapub.org/food>

Copyright © 2018 The Author(s). Published by Scientific & Academic Publishing

This work is licensed under the Creative Commons Attribution International

License (CC BY). <http://creativecommons.org/licenses/by/4.0/>

milk [6-7]. In this study relationship between human milk concentration, and hence sampling time and educational status of the lactating mothers are considered to show the variation pattern with them and an effort has been made to enlighten whether the elemental concentration of human milk varies with sampling time.

2. Methodology

2.1. Sample Collection and Preparation

Human milk samples were collected by hand expression from both breasts for about sixty two lactating mothers in different maternity centers of Dhaka during January 2006 to December 2007. The samples were collected twice in a day: one in the morning before breakfast and second in the afternoon after lunch to examine the effect sampling time on the elemental concentration for the same issue. The educational statuses of the corresponding mothers were known by questionnaire to find whether the human milk compositions vary with them.

Prior to collection, the nipple areas of the breasts were washed with soap and water, rinsed with distilled water and dried with a tissue. This greatly reduces the possibility of any contamination. Each milk sample was put in specially cleaned plastic bottles and thoroughly mixed together to ensure homogeneity from both breasts. They were immediately frozen and then freeze dried at about -85°C temperature and 133×10^{-3} mbar pressure [6]. After attaining a constant mass, the dried milk samples were grind to make fine powder and made into pellets and labeled and kept in desiccators. Before grinding a sample the crucible was properly cleaned with acetone every time to avoid contamination.

2.2. Method of Analysis

The proton induced X-ray emission (PIXE) technique was used to determine the essential and toxic elements in human milk used for this study. The main feature of PIXE method is to ionize an atom to produce characteristic X-ray and to detect them by the Si(Li) detector. The PIXE analysis provides high Z elements such as S, Cl, K, Ca, Fe, Cu, Zn, Ga, Br, Ru, Pb etc. and gives the accurate concentration of the most of the elements present in the samples with high accuracy.

In PIXE technique MAESTRO software was used to save the X-ray emission spectra and GUPIX with DAN32 interfacing software [8] was used for analyzing the spectra. The peak of the pulse height spectra gives information of an element and its concentration if it corresponds to the full-energy absorption peak of that element. The peak location is a measure of the photon energy and the peak area represents the number of photons. To identify the element, one has to measure the energy of the peak center accurately and then compare the energy with the PIXE yield database.

3. Results and Discussions

The mammary gland is capable of regulating concentrations of essential elements such as Cu, Fe and Zn in milk to protect the newborn infant against deficiency and excess of these elements. Our knowledge regarding most element concentrations, especially those of toxic metals, in breast milk, and how they are regulated, interact, or affected by maternal exposure, is however, limited. Despite many important advances in analytical techniques for trace element analyses in biological materials, such analyses are still commonly subject to significant errors due to mass interferences, high risk of contamination, and lack of suitable reference materials. Comparisons of breast milk concentration of elements across studies are hampered by differences in analytical performance, state of lactation and factors related to dietary habits and environmental concentrations. Therefore, literature data are inconsistent for many elements, and it is often not possible to conclude whether the differences are real (representing biological or dietary pattern variability) or artefacts arising from analytical difficulties [9]. This study investigated a wide variety of element concentrations of healthy mother's breast milk, using a highly sensitive analytical method which enables determination of very low concentrations of elements with high precision.

The Minimum Detection Limits (MDL) for this method usually depend on the matrix constituents of the sample. It is, therefore, necessary to keep attention of the MDLs to obtain quality results. Before doing any experiment, it is essential to validate the method accurately and is also important to demonstrate the quality of the data that are being used for decision making. It is necessary to present the data with the confidence that they really reflect the human health and thus environment [10-11] to convince the government or industries to take appropriate steps.

However, sometimes it is difficult to find the standards of similar matrix description to the field of respective samples for quality assurance practice. In such cases, validation and good knowledge of the fundamentals of the techniques are not necessarily sufficient to obtain good results; careful attention to details must be needed on the part of the analyst to ensure the quality of results.

3.1. Method of Validation

The validity of the method has been checked by analysing two standard samples: Standard Reference Material 1633b (Coal Fly Ash) from National Institute of Standard and Technology (NIST) and IAEA Milk Powder (A-11) from IAEA. Measurements were taken several times and the obtained values are presented in Table 1.

Almost maximum values found agreed with the certified values, indicating that the instrument performed well under the given conditions at the time of sample analysis. The average obtained result for both the cases agreed within $\pm 5\%$ of the certified values, which conforms the validity of

our method. The wide variation of concentration of the elements may be due to the different sample matrix. In addition, the accuracy and precision of the method was sufficient for the experiment. Figure 1 represents the PIXE spectra for standard reference material.

Table 1. Elemental concentrations of the Standard Reference Material

Elements	Coal Fly Ash		IAEA Milk Powder (A-11)	
	Certified value (ppm)	Measured Value (ppm)	Certified value (ppm)	Measured Value (ppm)
S	2075 ± 11	2059 ± 45	--	--
K	19500 ± 300	19163 ± 432	17200±1000	16845±1224
Ca	15100 ± 600	14962 ± 500	12900±800	13156±1421
Cl	--	--	9080±1740	8935±1512
Fe	77800 ± 2300	77575 ± 1500	3.65±0.76	4.53±1.4
Cu	112.8	118±8.45	0.84±0.165	1.23±0.32
Zn	210	220 ± 10	38.9±2.3	36.12±5.23
Br	140	145 ± 5	14	13.67±3.4
Rb	--	--	30.8±6.3	32±7.5

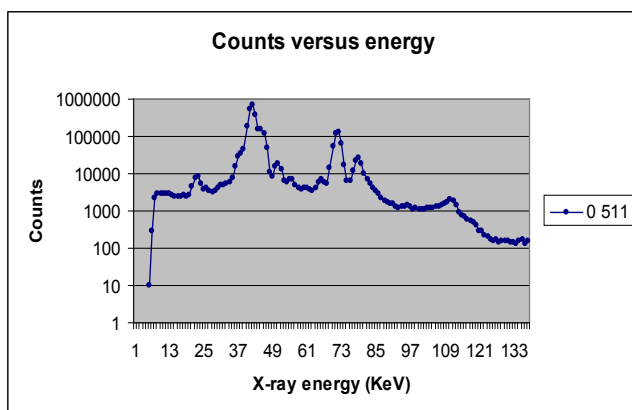


Figure 1. PIXE spectra for standard reference material

3.2. Effect of Sampling Time

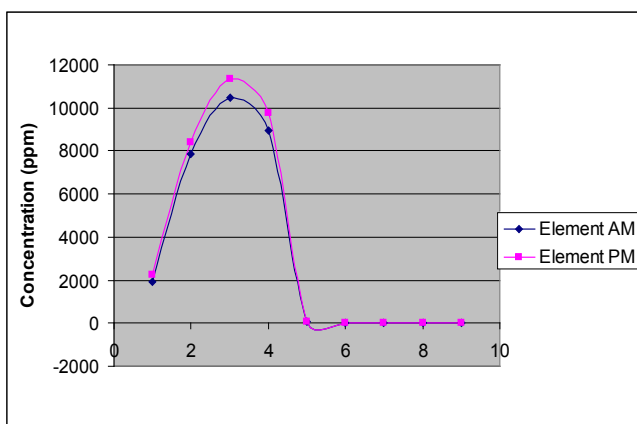


Figure 2. Variation of con. of human milk in terms of sampling time

The results of variation of trace elements in human milk samples with respect to sampling time are given in Figure 2. The samples were collected from the same mothers but at differing time; e.g. in the morning (AM) and in the afternoon (PM). It is clearly evident in Figure 2 that the concentrations of all the elements in the afternoon (PM) samples are higher than those in the morning samples (AM). Feeley et al. [12-13] also reported that elemental contents of the morning samples were significantly lower than those of the afternoon samples. This may be due to whole the night, the lactating mothers don't take food.

3.3. Effect of Educational Status of the Mothers

In almost all countries, child-death rates are inversely related to the level of maternal education. The more educated the mother, the healthier she and her child are likely to be. Nayak et al. [14] found that the socioeconomic factors of maternal education and household prosperity were associated with breast milk composition. Figure 3 shows the impact of mother's educational status on the concentrations of human milk constituents. Zn, Fe and Ca content in human milk varied significantly for the variation of mother's educational status. The concentration of Zn, Fe and Ca content of breast milk from mothers with SSC and above educational status was significantly higher than those of illiterate and primary educated mothers. Whereas, the concentrations of S, Cl, K, Cu, Rb and Br do not depend upon the variation of educational status of mothers. Though not significantly, Cu content tended to be higher for higher educational status mother.

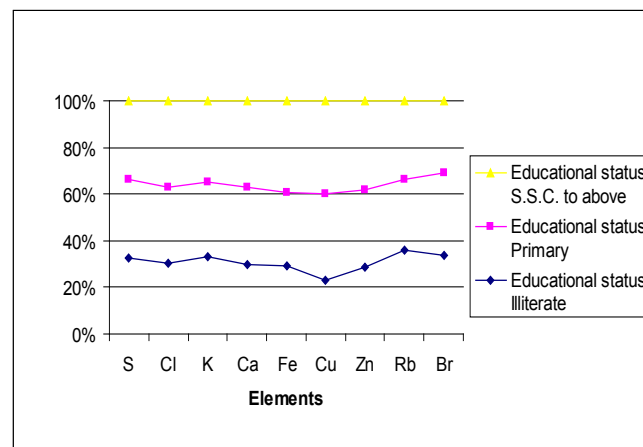


Figure 3. Effect of mother's education on human milk composition

4. Conclusions

Analysis of breast milk samples showed that the socioeconomic factors of maternal education and sampling time are associated with breast milk composition. Concentration levels of human milk for the elements of Zn, Fe and Ca are found to be significantly higher than those of illiterate and primary educated mothers. Whereas, those of

elements S, Cl, K, Cu, Rb and Br do not depend upon the variation of educational status of mothers. The concentrations of all the elements in the afternoon (PM) samples are higher than those in the morning samples (AM). Hence, this finding needs further investigations to give quantitative information about the elemental concentration and to obtain more information about its origin in relation to health consequences for mothers and children.

ACKNOWLEDGEMENTS

The authors wish to thank all the members of Accelerator Facilities Division for their continuous help during the course of this study. This work was partly financially supported by the Dutch Bangla Bank Foundation Limited, under the DBBL Fellowship Programme is also thankfully acknowledged.

REFERENCES

- [1] Kramer MS, Kakuma R, 2012, Optimal duration of exclusive breastfeeding. *Cochrane Database Syst Rev*, 8: CD003517.
- [2] A K M Fazlul Hoque, 2000, Analytical Application of Charged Particle Induced Nuclear Reactions for Low Mass Elemental Analysis. PhD Dissertation, Physics Department, Jahangirnagar University.
- [3] M. Khaliquzzaman, A. H. Khan, S. K. Biswas, 1999, Nature and the extent of airborne particulate matter pollution in urban and rural areas of Bangladesh during 1993-98, Technical report, AECD/AFS-CH/9-50.
- [4] A. Islam, 2000, Studies of Some Trace Elements in Bangladesh Agro-ecosystem, Ph.D. Thesis, Chemistry Department, University of Dhaka, Dhaka.
- [5] Parker, J. and D. Pearce, 2001 Microfinance, Grants and Non-Financial Responses to Poverty Reduction: Where Does Micro credit Fit? Consultative Group Focus Note No. 20: Washington, DC.
- [6] S. O. Olabanji, M. C. Buoso, D. Ceccato, A. M. I. Haque, R. Cherubini and G. Moschini, 1996, *Nucl. Instr. Meth. in Phys. Res. B* 109/110, 258-261.
- [7] Natural Resources Defense Council. Healthy Milk, Healthy Baby—Chemicals: Lead, Mercury, Cadmium, and Other Metals. 2014. <http://www.nrdc.org/breastmilk/lead.asp>.
- [8] S. A. E. Johanson and J. L. Campbell, 1988, PIXE: A Novel Technique for Elemental Analysis, Wiley, Chichester.
- [9] K. L. Björklund, M. Vahter, B Palm, M. Grandér, S. Lignell and M. Berglund, 2012, Metals and trace element concentrations in breast milk of first time healthy mothers: a biological monitoring study, *Environmental Health*, 11, 92.
- [10] Proficiency testing by interlaboratory comparisons Part 1, 1997: Development and operation of proficiency testing schemes, Guide 43-1, ISO.
- [11] Michael Gray, Shay Phillips and Robert McMahon, 2014, Simultaneous determination of nutritional elements in human milk by inductively coupled plasma-mass spectrometry, *The FASEB Journal*, 28 (1), Supplement 623.4.
- [12] R. M. Feeley, R. R. Eitenmiller, J. B. Jones and H. Barnhart, 1983, *American J. Clin. Nutr.*, 37, 443- 448.
- [13] Nicholas J. Andreasa, Beate Kampmannac, Kirsty Mehrling Le-Doare, 2015, Human breast milk: A review on its composition and bioactivity, 91 (11), 629-635.
- [14] U Nayak, S Kanungo, D Zhang, E. Ross Colgate, Marya P. Carmolli, A Dey, M Alam, B Manna, R K Nandy, D R Kim, D K Paul, S Choudhury, S Sahoo, William S. Harris, Thomas F. Wierzb, T Ahmed, Beth D. Kirkpatrick, R Haque, William A. Petri Jr, Josyf C. Mychaleckyj, 2017, Influence of maternal and socioeconomic factors on breast milk fatty acid composition in urban, low income families, *Maternal & Child Nutrition*, John Wiley & Sons Ltd, 13:e12423.